

# Light Harvesting as a Simple and Versatile Way to Enhance Brightness of Luminescent Sensors

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The emissive output of indicator dyes in luminescent sensors can be amplified by the addition of antenna dyes with a higher brightness. The highly concentrated antenna dye molecules absorb the excitation light and transfer the energy to an indicator dye. This harvesting of light makes thin sensor layers (thickness <500 nm) and nanometer sized sensor particles with exceptionally high brightness and compatible with the most powerful LEDs available. The performance of sensor layers of ~250 nm thickness employing light harvesting was investigated and compared with established sensors. The principle is demonstrated for oxygen and ammonia sensors. An overview of possible application of light harvesting to various reagent mediated optical sensing schemes is given.

Signal enhancement is one of the major tasks in the improvement of luminescent based sensors. Reasons for that can be found in the need to improve the signal-to-noise ratio, to minimize the influence of background fluorescence or ambient light, to reduce the thickness of sensing films, and to apply simple optoelectronic readout systems. Various strategies are known to improve the signal intensity. Apparently, the design of fluorescent indicators with high brightness (defined as the product of molar absorption coefficient and quantum yield) is straightforward yet limited by synthetic challenges. Alternatively, modifications of the optical system were investigated including the use of parabolic collectors to detect the supercritical angle fluorescence,<sup>1,2</sup> conical optics in fiber optic microsensors,<sup>3,4</sup> or patterned substrates.<sup>5</sup> Methods based on surface enhanced fluorescence are able to increase the brightness for dyes with low molar absorption coefficients and quantum yields up to ~20%, but the enhancement depends strongly on the distance to the metal surface (short distances result in quenching).<sup>6–8</sup> Another method applied is the increase

of optical path length by adding scattering centers to the sensing layers.<sup>9,10</sup> However, the most efficient scatterer titanium dioxide acts also as photocatalyst and often reduces the photostability of the sensors. One of the simplest strategies to increase the amount of absorbed light by increasing the indicator dye concentration is compromised by luminescence self-quenching and by long response times for low analyte concentrations. Our method uses light harvesting to increase the brightness of reagent mediated sensors.

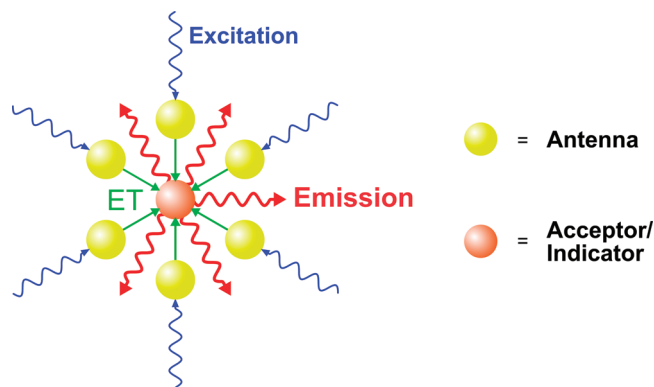
Light harvesting is well-known from nature's photosynthesis and is used in dye sensitized solar cells to improve the excitation efficiency.<sup>11</sup> Few examples of light harvesting used in optical sensors are reported including conjugated polymers,<sup>12</sup> dendrimeric systems,<sup>13</sup> and fluorescent labels using phycobilly proteins.<sup>14</sup> In this paper we present the application of this principle in reagent mediated optical sensors to amplify the emissive output of indicator dyes. The basic idea is to increase the amount of absorbed light and emitted light by adding an inert dye with a higher brightness. The principle of light harvesting utilizes energy transfer (ET) from donor to acceptor dye molecules.<sup>15</sup> The donor dye acts as antenna for the excitation light and transfers the energy to the analyte sensitive acceptor dye (indicator) as shown in Figure 1. The concentration of the donor is chosen much higher than the concentration of the acceptor molecules. Consequently, one acceptor molecule absorbs the energy harvested of several donor molecules in close proximity. This results in a highly efficient absorption of the excitation light and in increased emission of the acceptor dye compared to the intrinsic excitation.

This method to enhance brightness of the fluorescent sensor is simple and flexible. Light harvesting can be applied to established sensing schemes and materials without a significant influence on the dynamic range, selectivity, and sensitivity. Figure

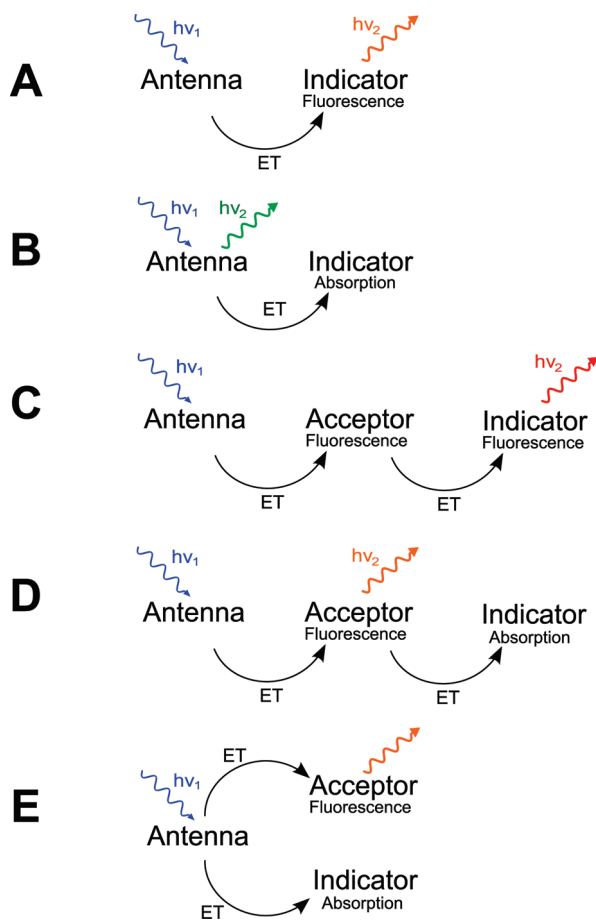
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**Figure 1.** (a) Principle of light harvesting as applied in optical sensors. Antenna dye molecules collect light and transfer the energy to an analyte-sensitive acceptor (indicator) dye.



**Figure 2.** Possible sensing schemes employing light harvesting for signal amplification (A–E) and Stokes-shift extension (C–E).

2 summarizes the various possible schemes for signal enhancement by light harvesting: (A) The antenna dye collects light and transfers energy to a fluorescent indicator; (B) the antenna transfers energy to an absorption indicator. With dependence on the absorption properties of the indicator (related to the presence and absence of the analyte), the fluorescence signal of the antenna dye is detected. In addition to signal enhancement, light harvesting can extend the Stokes' shift and facilitate the separation of excitation and emission light. This is summarized in the following schemes: (C) Light is collected by an antenna dye and transferred in a cascade via a fluorescent acceptor dye to the indicator. (D)

The collected light is transferred to an acceptor with overlapping spectra of an absorption indicator. The emission results from the fluorescent acceptor. (E) The energy is transferred to an absorption indicator and another fluorescent acceptor. If the absorption of the indicator is high, the fluorescent signal detected will be low and vice versa.

Simple brightness enhancement is particularly attractive for ultrathin sensor films (below 1  $\mu\text{m}$ ) and nanometer-sized sensor particles to obtain sufficient performance. Additional benefits of light harvesting systems are an extended Stokes' shift and the possibility to tune the excitation to wavelengths where bright and inexpensive light sources are available. For demonstration, we present the use of coumarin antenna dyes to enhance the performance of luminescent oxygen sensors and ammonia sensors based on pH-indicators.

## EXPERIMENTAL SECTION

**Materials.** Coumarin 545T (C545T), eosin ethylester (eosin), sulforhodamine B (SRB), bromophenol blue (BPB), cellulose acetate propionate and polystyrene were purchased from Aldrich ([www.sigmaaldrich.com](http://www.sigmaaldrich.com)). Platinum(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorophenyl)-porphyrin (PtTFPP) was purchased from Frontier Scientific (Carnforth, United Kingdom). Silicone (PP2-RG01) was purchased from ABCR ([www.abcr.de](http://www.abcr.de)).  $\text{Ir}(\text{C}_5)_2(\text{acac})$  was synthesized in our laboratory. Aqueous ammonia solutions were prepared by dissolving ammonium chloride in a 100 mM sodium phosphate buffer at pH 7.

**Preparation of Sensor Layers.** Antenna dyes, indicator dyes, and polymer matrix were dissolved in chloroform or acetone. The "cocktails" were knife-coated onto a dust-free polyester support, and the solvent was allowed to evaporate under ambient air. The sensor thickness was calculated from absorption measurements. Sensing films of nanometer thickness were coated on glass slides. The ammonia sensor layers were covered with a 5% (w/w) silicone solution in *n*-hexane to obtain a 1  $\mu\text{m}$  thick silicon layer.

**Spectral Measurements.** Emission and excitation spectra were acquired on a Hitachi F-7000 fluorescence spectrometer ([www.inula.at](http://www.inula.at)) equipped with a red-sensitive photomultiplier tube (PMT) R 928 from Hamamatsu ([www.hamamatsu.com](http://www.hamamatsu.com)). The emission spectra were not corrected for the sensitivity of the PMT. Absorption spectra were measured at a Cary 50 UV-vis spectrophotometer ([www.varian.com](http://www.varian.com)). Absorption spectra of dissolved dyes were obtained in chloroform or water.

Luminescence lifetime was determined by phase shifts measurements with a two-phase lock-in amplifier SR830, Stanford Research Inc. ([www.thinksrs.com](http://www.thinksrs.com)). The light harvesting and  $\text{Ir}(\text{C}_5)_2(\text{acac})$  containing sensor films were excited with the light of a blue light-emitting diode (LED) ( $\lambda_{\text{max}} = 470 \text{ nm}$ , [www.roithner-laser.com](http://www.roithner-laser.com)), which was sinusoidally modulated at a frequency of 5 and 20 kHz, respectively. The sensing films composed of solely PtTFPP were excited with a UV-LED ( $\lambda_{\text{max}} = 395 \text{ nm}$ , [www.roithner-laser.com](http://www.roithner-laser.com)), which was sinusoidally modulated at a frequency of 5 kHz. A bifurcated fiber bundle was used to guide the excitation light to the sensor film and to guide the luminescence to a PMT (H5701 02, Hamamatsu, [www.hamamatsu.com](http://www.hamamatsu.com)) after passing a RG645 or OG550 (Schott, Mainz, Germany) glass filter. Solutions were deoxygenated by adding an amount of 100  $\mu\text{L}$  of glucose oxidase solution ( $c =$

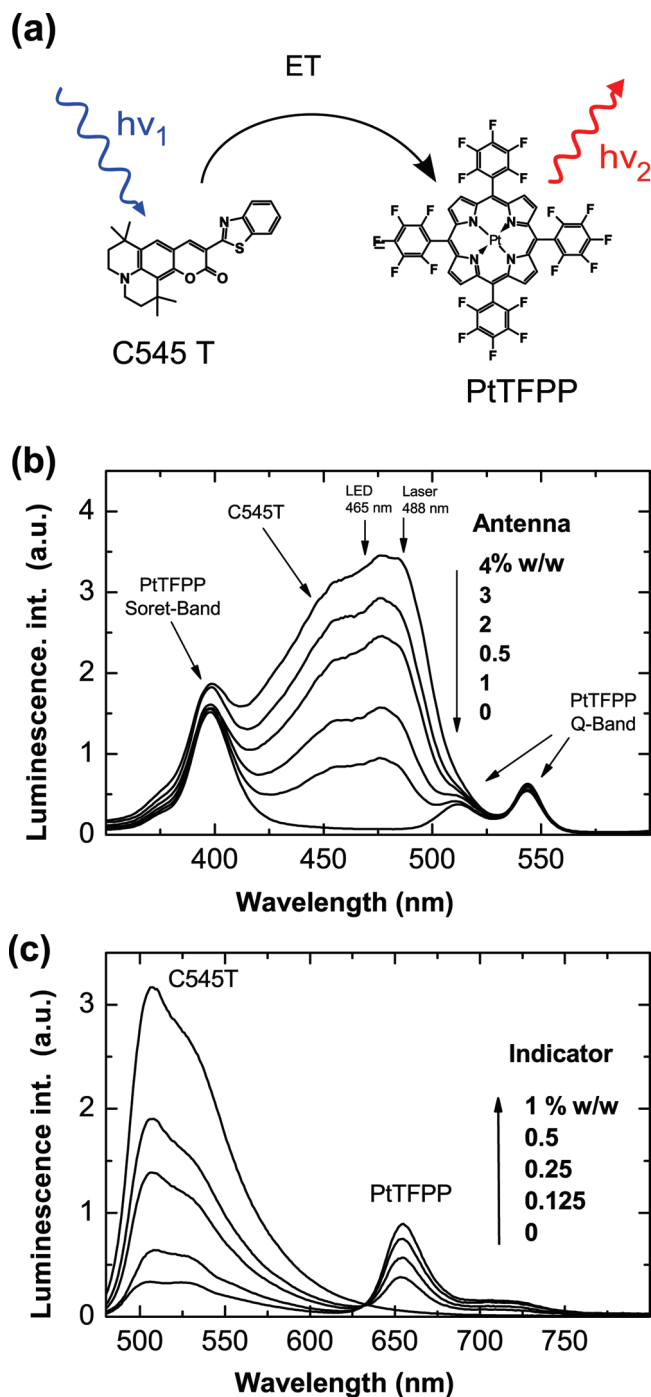
10 mg/mL) to 5 mL of glucose solution. The concentration of glucose was 0.25 mol/L.

## RESULTS AND DISCUSSION

The application of light harvesting in fluorescent sensor films with a short optical path-length ( $<1 \mu\text{m}$ ) requires antenna dyes to meet the following criteria: (a) high molar absorption coefficient ( $\epsilon > 30\,000 \text{ L mol}^{-1} \text{ cm}^{-1}$ ); (b) good solubility in the host polymer (2% w/w) to absorb as much excitation light as possible. Note that high dye concentrations ( $>1\%$  w/w) commonly result in self-quenching due to aggregation or recrystallization of the dye molecules; (c) a high emission quantum yield ( $>0.9$ ); (d) an overlap of the emission spectra with absorption spectra of the indicator (acceptor) dye; and (e) high photostability. Potential candidates can be found in the classes of coumarin or perylene dyes. Among them, the coumarin C545T was found to fulfill most of these requirements, particularly the excellent solubility in polymeric matrixes due to the bulky structure together with the spectral compatibility with blue LEDs (465 nm) and Ar–Ne laser (488 nm). In addition, C545T is commercially available at low prices.

Brightness enhancement of oxygen sensors is demonstrated employing C545T and PtTFPP (a widely used oxygen indicator with excellent photostability).<sup>16</sup> Figure 3a illustrates the sensing scheme of this combination. Figure 3b shows the excitation spectra recorded at 650 nm (emission maximum of PtTFPP) of polystyrene sensing films containing varying concentrations of the antenna dye (0–4% w/w, 0–93 mmol/kg) and a constant indicator concentration (1% w/w, 8.6 mmol/kg). Higher antenna dye concentrations result in an increase of absorbed light and a raise of fluorescence. The band of C545T between 440 and 490 nm increases, while the bands of PtTFPP do not change significantly. In practice, visible excitation is preferable compared to UV excitation due to a lower level of background fluorescence. Herein, a blue light excitation of the antenna dye yields in signal amplification of approximately 5.5 (calculated by the ratio of the signal at 475 and 545 nm). Additionally, blue LEDs (465 nm) are the most powerful available, which is an additional amplification factor. The sensitivity to oxygen is not influenced by the added antenna dye as the quantum yield of the indicator dye does not change significantly which is indicated by the constant signal of the Q-band in the excitation spectra at 545 nm. The efficiency of the ET depends on the concentrations of PtTFPP as shown in Figure 3c. The intensity of the remaining green fluorescence of C545T can be tuned and used for ratiometric measurement at two wavelengths.

**Performance of Oxygen Sensor Films of Nanometer Thickness.** The increase of sensor brightness employing light harvesting enables the reduction of sensor film thickness and response time. Commonly, the sensor film thickness is  $\sim 5 \mu\text{m}$ , when indicators with moderate brightness are employed. Light harvesting enables sensor films with a thickness below  $1 \mu\text{m}$ , which provide a sufficient signal due to the increased brightness. Light harvesting sensing films composed of C545T (4% w/w) and PtTFPP (1% w/w) with a thickness of 450 nm absorb  $\sim 30\%$  of the excitation light at 470 nm as evidenced by absorption measurements. The diffusional barriers for the diffusion limiting quenching



**Figure 3.** (a) Scheme illustrating light harvesting as applied to oxygen sensors. (b) Excitation spectra recorded at 650 nm of polystyrene sensor films in a thickness of  $\sim 1.5 \mu\text{m}$  at varying concentration of the antenna coumarin 545T and constant concentration of the acceptor PtTFPP (1% w/w). (c) Emission spectra excited at 465 nm of polystyrene sensor films in a thickness of  $\sim 3 \mu\text{m}$  at a varying concentration of the acceptor and constant concentration of the antenna dye (2% w/w).

process in such thin sensor films are dramatically reduced. This results in ultrashort response times. The response time  $t_{95}$  on going from air to oxygen-free solution is 0.8 s for the light harvesting-enhanced sensing film, while standard oxygen sensing films (thickness  $5 \mu\text{m}$ ) show a response time  $t_{95}$  of  $\sim 10$  s (see Supporting Information).

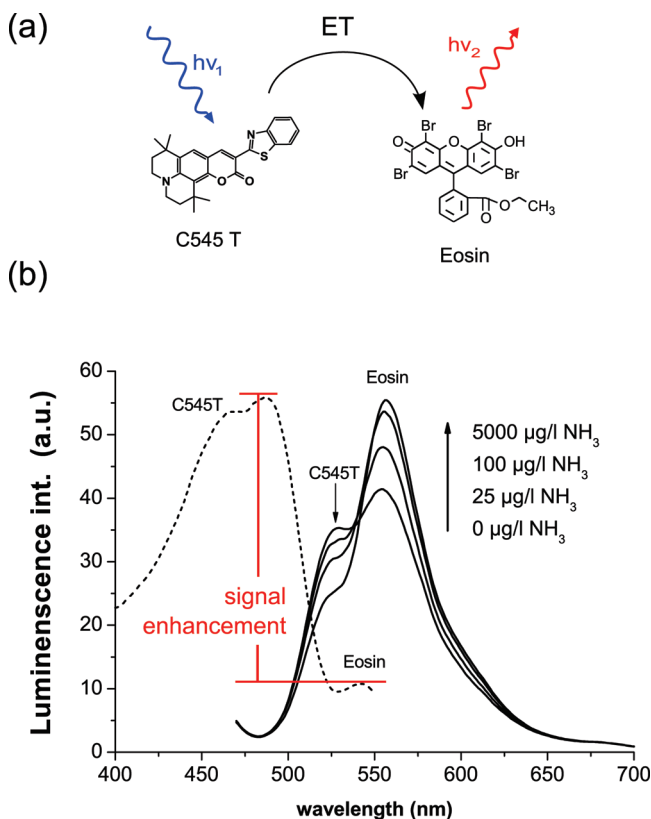
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**Table 1. Performance Data of Oxygen Sensing Films of ~250 nm Thickness**

sensor components (w/w)	$\tau_0$ ( $\mu$ s)	$\tau_0/\tau$	drift of intensity <sup>a</sup> at 200 hPa O <sub>2</sub> (% h <sup>-1</sup> )	$\Delta$ pO <sub>2</sub> <sup>a,b</sup> at 200 hPa O <sub>2</sub> (hPa h <sup>-1</sup> )
C545T (4%)/PtTFPP (2%)	48.1	3.0	17.5	3
PtTFPP (2%)	49.0	2.9	0.6	1
Ir(C <sub>5</sub> ) <sub>2</sub> (acac) (2%)	5.9	1.5	8.3	17

<sup>a</sup> Acquired under continuous illumination of sensing films at 25 °C.

<sup>b</sup> Calculated from phase shifts measurements.

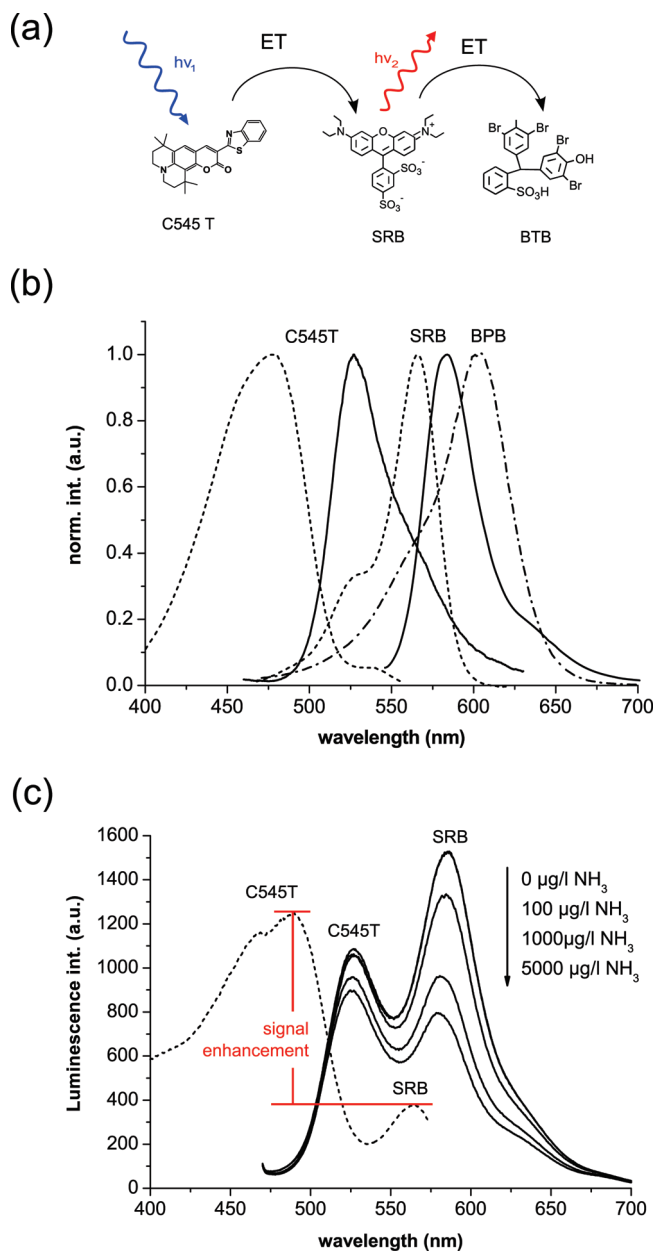


**Figure 4.** (a) Scheme illustrating light harvesting as applied to an ammonia sensor making use of a fluorescent pH-indicator (eosin). (b) Excitation (dashed line) and emission (solid line) spectra of a sensor film containing 1% w/w C545T and 2 mmol/kg eosin (thickness of  $\sim 1 \mu$ m) exposed to various concentration of dissolved ammonia.

The performance of light harvesting sensing film nanometer thickness is compared with oxygen sensing films making use of the standard indicator PtTFPP and the ultrabright indicator Ir(C<sub>5</sub>)<sub>2</sub>(acac). The latter was recently reported by our group, shows extraordinary brightness, and is excitable with blue LEDs.<sup>17</sup> The performance data of the investigated sensing films are summarized in Table 1.

The light harvesting film shows a higher sensitivity (expressed as  $\tau_0/\tau$ ) than the Ir(C<sub>5</sub>)<sub>2</sub>(acac) based film and a comparable sensitivity as the standard PtTFPP sensing film. The lifetime of the light harvesting sensing film is slightly lower than the lifetime the PtTFPP film. The photostability of any reagent mediated optical sensor is of high importance and is of particular interest for long operation times. PtTFPP is known

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**Figure 5.** (a) Scheme illustrating light harvesting applied to an ammonia sensor making use of an energy transfer cascade from C545T to the fluorescent acceptor (SRB) and a nonfluorescent pH-indicator (BPB). (b) Normalized absorption (dashed line) and emission (solid line) spectra of the used dyes. (c) Excitation (dashed line) and emission (solid lines) spectra of the sensing film containing 1% C545T, 0.2% SRB, and 2 mmol/kg BPB (thickness  $\sim 1 \mu$ m) exposed to various concentration of dissolved ammonia.

to produce singlet oxygen in aerated samples that can result in degradation of the sensor components. Therefore photodegradation rates were acquired under continuous illumination of the sensing films exposed to synthetic air with an LED at a constant temperature of 25 °C. It should be mentioned that in practice, sensors are not operated under continuous illumination. In comparison, the light harvesting enhanced sensing film containing C545T is less photostable than the Ir(C<sub>5</sub>)<sub>2</sub>(acac) containing film, while the PtTFPP film is not perceptibly influenced. Although the luminescence intensity signal of light harvesting sensing film is affected by photodegradation, the lifetime is minimally influenced. Note that lifetime phase shift

measurements are more reliable and established in practical sensor applications. The error in the determination of oxygen (calculated from lifetime measurements) for the light harvesting sensing film is comparable to the error of the standard PtTFPP film, while the film containing Ir(C<sub>5</sub>)<sub>2</sub>(acac) causes a rather high error.

**Ammonia Sensors.** The principle of light harvesting is also applied to trace level ammonia sensors based on pH-indicators.<sup>18,19</sup> The examples demonstrate brightness enhancement, Stokes'-shift extension, and the possibility of referencing by two wavelengths rationing. Figure 4a illustrates the first example. The antenna C545T collects light and the energy is transferred to the acceptor (eosin). Figure 4b shows the excitation and emission spectra of a sensing film containing 1% (w/w) C545T and 2 mmol/kg eosin. On exposure to ammonia the emission band of eosin ( $\lambda_{\text{max}} = 560$  nm) increases, and the band of C545T decreases ( $\lambda_{\text{max}} = 525$  nm). The signal is amplified by a factor of 5 calculated from the signal of the excitation spectra at 470 and 540 nm representing the signals of C545T and eosin, respectively.

The second ammonia sensor makes use of an energy transfer cascade as shown in scheme D of Figure 2. The energy is transferred from C545T to a SRB (fluorescent acceptor) and subsequently to BPB (nonfluorescent pH-indicator (see parts a and b of Figure 5)). The response to dissolved ammonia is shown in Figure 5c. The emission band of SRB ( $\lambda_{\text{max}} = 585$  nm) decreases with increasing analyte concentrations. With this scheme, the Stokes'-shift is enlarged to 96 nm and a 4-fold signal intensity enhancement is achieved.

## CONCLUSION

Light harvesting is a simple yet versatile method to enhance the sensor performance of reagent mediated luminescent sensors. The signal is amplified, and the excitation can be adjusted to the most powerful and cheap light sources without changing the sensor properties. In an analogous manner, the performance of

reagent-mediated optical sensors for other analytes such as pH, carbon dioxide, and ions can be improved. The presented oxygen sensor shows comparable brightness as the brightest sensor reported, and also a stable lifetime signal. In addition, the light harvesting sensor has a larger Stokes'-shift and a red luminescence ( $\lambda_{\text{max}} = 650$  nm). At these wavelengths background fluorescence of the sample is marginal. The employed coumarin dyes are suitable for the excitation with blue light. Other antenna dyes or combinations of dyes in an ET-cascade enable an excitation at different wavelengths and the use of other indicator dyes. Alternative antenna dyes are, e.g., perylene dyes, which have the additional benefit of good photostability. The combination of several antenna dyes in an ET-cascade enables the collection of light over a broad range of a few hundred nanometers (see Supporting Information). Sensor layers employing light harvesting of nanometer thickness respond in real-time and make monitoring of fast processes possible. Moreover, thin sensing layers have better adhesion properties increasing the long-term stability. Light harvesting can also be applied in (nano) sensor particles (see Supporting Information) yielding bright tools in fluorescence microscopy or for lab-on-a-chip (microfluidics) applications.

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## SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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